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14. ABSTRACT
Prostate carcinoma is the most common form of cancer in American men. The etiology of prostate cancer is currently unknown. It is known that during progression from low to high-grade carcinoma, the spectrum of cytologic, biological, and genetic features changes. We believe that these changes may be a result of defects in the centrosome, an essential organelle that organizes spindle poles during mitosis and has important roles in cell proliferation, cell polarity, and genetic stability. We have shown that centrosomes are defective in prostate carcinoma and pre-invasive lesions. We also showed that the essential centrosomal protein pericentrin is elevated in both pre-invasive prostate lesions and invasive prostate tumors, induces cancer like lesions when overexpressed and binds AKT, PKA and PKC. In this proposal we will follow up on these observations by: examining pericentrinjs oncogenic potential after disrupting its interaction with the kinases AKT, PKA and PKC and determine whether other oncogenes or tumor suppressors act synergistically or antagonistically with pericentrin in prostate cancer.

15. SUBJECT TERMS
Centrosome defects, genetic instability, prostate cancer progression

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Introduction

The work in this proposal is designed to investigate the role of pericentrin as an oncogene and centrosme-damage-checkpoint protein in prostate normal and cancer cells. Pericentrin is a centrosome protein involved in organizing mitotic spindles to ensure proper chromosome segregation (*1*). The two poles of mitotic spindle during cell division are formed by a pair of centrosomes, each of which is composed of an amorphous matrix and two microtubule barrels called centrioles (*2*). Each centriole duplicates once per cell cycle and requires 1.5 cell cycles to mature (*3*). Previous work from our laboratory and another group revealed that centrosome defects were present in nearly all prostate cancers (*4-6*). In other work we showed that modulating pericentrin levels by over-expression or siRNA-mediated depletion can lead to a variety of centrosome defects, including centrosome amplification, spindle defects, the loss of centrioles/centrosomes and the loss of cilia (*7, 8*). We later characterized a novel centrosome-damage-checkpoint (CDC) in normal human diploid cells, which can be activated upon pericentrin depletion (*8*). The activation of this checkpoint also requires functional p53 and p38 and leads to G1 arrest (*8*). In cancer cells with defective regulatory pathways (p53 or p38), we found that the CDC activation induced cancer-cell-specific cell death, but we did not look at cell cycle arrest. Because this "poisoning" strategy is highly selective, we planned to explore its applicability to treat prostate tumors with an abrogated p53 pathway (e.g. PC3 and DU-145).

Body

Over the last year, I tested whether the CDC induced G1-arrest in normal prostate cells and in p53-functional cancer cells, but apoptotic death in p53-abrogated cancer cells. While cell death was observed it was not substantial even after long period of protein depletion. However, during the course of these studies, we noticed that all prostate cells treated with pericentrin siRNA were more likely to fail in cytokinesis at first and become polyploid and aneuploid later. These severe genomic changes are known to exacerbate the tumor progression and potentially drive tumorigenesis from benign to malignancy (*9*).

It is possible that this mechanism of cell division failure could account for centrosome defects and amplification that we have consistently observed over the last several years in prostate tumors and tumor cell lines (*5-7*). In fact, we believe that cytokinesis failure may be the primary mechanism by which centrosome defects and aneuploidy develop during prostate tumorigenesis. When a prostate cell fails cell division, it instantly becomes tetraploid (4N) with supernumerary centrosomes (twice the normal number).

In the next year, I will explore the exciting possibility that pericentrin depletion leads to cytokinesis failure. Toward this goal, I will examine the mechanism of cytokinesis failure, identify pericentrin interacting proteins and understand how they function during cytokinesis. These studies will be another step toward the development of potential therapy for prostate cancer progression (centrosome defects are present as early as PIN lesions and in aggressive tumors. Cytokinesis is a therapeutic target that is rather novel and not a focus of most research groups or biotechnology companies.

Toward the goal of this proposal, we recently identified centriolin as a pericentrin-interacting protein. Centriolin localizes to the midbody (*10, 11*), a complex protein structure composed of hundreds of proteins that is formed at the intercellular bridge that connects the two daughter cells late in cytokinesis (*12*). The bridge needs to be resolved at the midbody by centriolin-involved asymmetric vesicle targeting (*11, 12*) as well as rearrangement of cytoskeletal elements and/or signaling proteins. Perhaps most exciting is the observation that CD133, a pan-stem cell marker, is highly enriched at midbodies (*14*).

The finding on midbodies described above suggest that these structures may have unexpected functions, some possibly unrelated to cytokinesis. Consistent with this idea, I recently observed that midbody (MB) is accumulated in prostate cancer cells (Fig. 1); we call these post-division midbodies midbody derivatives (MB^ds). In contrast, MB^d did not accumulate in normal prostate epithelial cells (gift of Dr. William C. Hahn, Harvard Medical School, Dana-Farber Cancer Institute). In addition, we showed that MB^d-containing cells were found in sections from human prostate tumors. Further studies demonstrated that MB^d were found in a number of different cancer cell lines but rarely found in normal dividing, differentiating, or telomerase-immortalized cells (Fig. 1). MB^ds were also found in stem cells in many human and mouse tissues (e.g. the bulge of hair follicles, the spermatogonia layer of seminiferous tubules. Fig. 3) as well as human embryonic stem cells (hESCs, H1 and H9) and mouse somatic cells induced to become stem cells (induced pluripotent stem cells. Fig. 2). Based on these surprising and provocative results, we propose that the MB^d-accumulating cells may have stem cell qualities and may represent "prostate cancer stem cells" or "prostate cancer initiating cells". These results would be consistent with the stem cell theory of prostate cancer (*15*).

Our previous results suggested that the inheritance and accumulation of MB^ds by one cell requires asymmetric MB^d asymmetric vesicle targeting to the intercellular bridge (*11*). To further test the mechanism of this asymmetric inheritance, I showed that the two daughter cells can be differentiated based one the age of the centrosome ages (Fig. 4). Like DNA, the centrosome is replicated in a semi-conservative manner resulting in an old copy and a younger copy. Using GFP-tagged centrin 1, a centrosome protein able to discern centrosome ages and time-lapse imaging, I found that the daughter cell with older centrosome preferentially received MB^ds (Fig. 5) and accumulated MB^ds in successive divisions.

We next asked where supernumerary MB^ds resided in the cell. The traditional view (from the 1960-70s) is that they are either degraded extracellularly or jettisoned from the cell. However, these is not we observed. Using wheat germ agglutinin to delineate plasma membrane, we showed that MB^ds are founded beneath the cell membrane within the cytoplasm (Fig. 6). As far as I know, we are the first group to unequivocally show the intracellular localization of MB^ds.

We next examined the fate of MB^ds. Because they are rarely accumulated in normal cells, but regularly accumulated in prostate cancer cells (and other cancer cells), we reasoned

that the degradation may play a role in the fate of these structures. To test this, we synchronized normal diploid cells (hRPE1) to facilitate synchronous generation of MB^ds during mitosis. We found that ~70% of MB^ds were within lysosomes 3 hours after release from mitosis (Fig. 7). With time, the number of MB^ds in lysosomes decreased. In contrast, cancer cells' MB^ds did not appear to be delivered to lysosomes and thus appear to escape from or be delayed in degradation. Currently, we are testing whether inhibiting lysosomal enzyme activity would increase the number of MB^{ds} in lysosomes in the normal cells. In addition, we are trying to understand mechanisms of MB^d degradation and how a subpopulation of prostate cancer cells accumulates MB^ds. Also under investigation is the question of whether accumulated MB^ds can affect cellular physiology, with an emphasis on their contribution to stem cell/cancer "stem" cell biology

Key research accomplishments

*Midbody derivatives accumulate in putative (cancer) stem cells and stem cell-niches.

*Cells with older centrosomes inherit midbody derivatives.

*Midbody derivatives can be degraded intracellularly.

Reportable outcomes

MANUSCRIPTS:
Asymmetic inheritance and accumulation of midbodies in stem cells. (in preparation)

PRESENTATIONS:
Midbody derivatives: novel structural prostate cancer stem cell marker (IMPaCT meeting, Atlanta, Georgia)

Midbody accumulate in putative stem cells (ASCB conference, venue)

CELL LINES:
Cell lines (hRPE, HeLa and PC3) express GFP-Mklp1 were established to study MB dynamics and subcellular localization post-cytokinesis.

Cell line (MCF7 and HeLa) expresses GFP-Cep55 were established to study MB dynamics and subcellular localization post-cytokinesis.

Cell line (RPE1) expresses mCherry-Cep55 was established to study MB dynamics and subcellular localization post-cytokinesis.

Cell line (MCF7) express Centrin 1-GFP were established to study how centriole age affects the MB inheritance.

Conclusion:

Progress during the last year includes three important progresses. Firstly, we found that post-mitotic MB^ds are not disassembled extracellularly or actively jettisoned by the daughter cells. Instead, they appear to be degraded *inside* cells by lysosomes in normal diploid cells and remain free in the cytoplasm of prostate cancer cells. This entire pathway has been ignored for decades and we are the first group to systematically investigate this process. Secondly, we demonstrated that putative prostate cancer "stem/initiating cells" (and other cancer cells) accumulate MB^ds in contrast to normal diploid cells that degrade them in lysosomes. This difference could be exploited to target prostate tumor cells versus normal prostate epithelial cells. Thirdly, we have established a link between MB^ds inheritance and the age of centrioles. This finding could contribute to the mechanism of asymmetric vesicle delivery at the very end of cytokinesis, which appears to be a complex process with contributions from many pathways (*12*).

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APPENDIX

Fig. 1

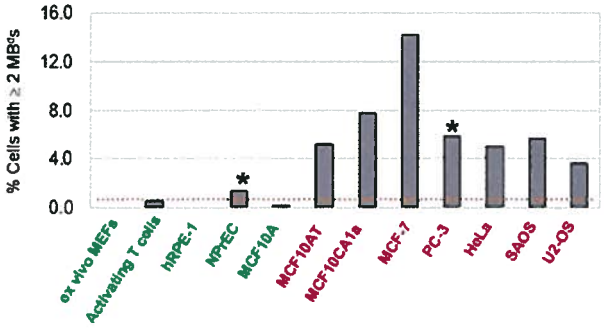

Fig. 2

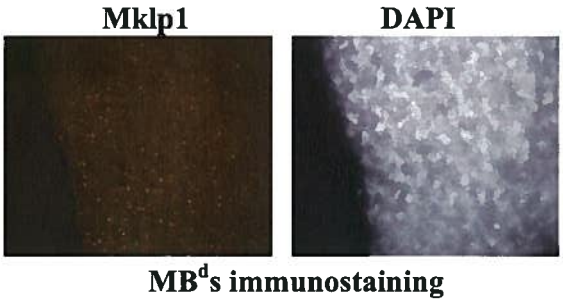

Fig. 3

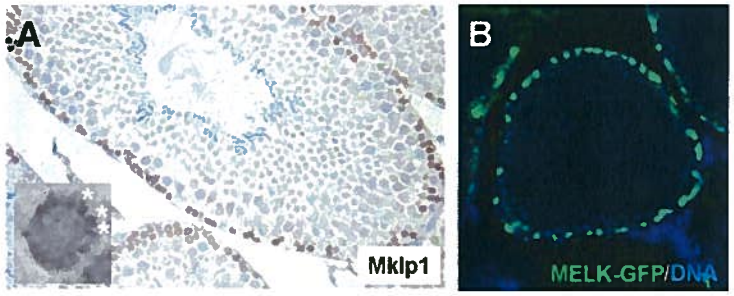

Fig. 4

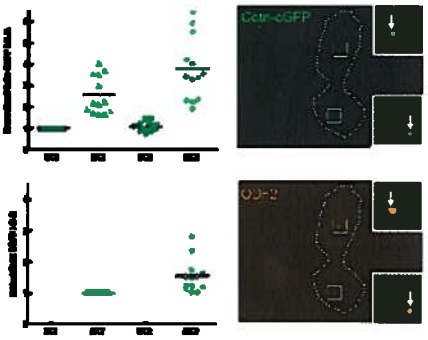

Fig. 5

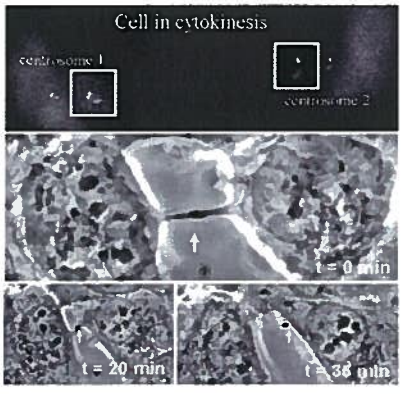

Fig. 6

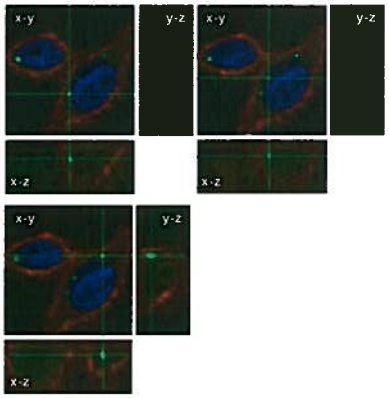

Fig. 7

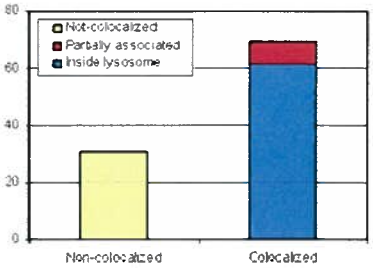

Figure Legends. Fig. 1. Quantification of multiple MB^d accumulation in different types of cells. Only a subset of cancer cells accumulates MB^{d}s. The dividing primary cells and normal cell lines are colored in green and cancer cell lines are in red. Prostate cells are marked by asterisks. **Fig. 2.** Mouse induced pluripotent stem cells (iPS) accumulate MB^{d}s but mouse embryonic fibroblasts do not (see Fig. 1). **Fig. 3.** (A) MBds are enriched in the spermatogonia layer of the testes, a well-defined stem cell-niche. (B) The niche is visualized by MELK-GFP, a stem cell-specific transcriptional factor. **Fig. 4**. The brighter Centrin-eGFP (Cetn-eGFP) labeled mother centrioles (MC) possess more MC-specific proteins, including cenexin/ODF2 and centriolin (not shown). **Fig. 5.** The representative snapshots during time-lapse imaging to show that MB^d is inherited to the daughter cell with older centrosome. **Fig. 6**. MB^{d}s are found reside within cytoplasm. MB^{d}s, WGA and nuclei are pseudo-colored in green, red and blue. **Fig. 7.** MB^{d}s localization in post-cytokinetic hRPE1 cells. Mitotic hRPE1 are shake-off and released. Three hours after replating, ~70% MB^{d}s are within or colocalized with lysosomes.